REMARKS

In view of the following Remarks, the Examiner is requested to withdraw the rejection and allow claims 14, 15, 25, 26, and 28-34, the only claims pending in this application and currently under examination.

FORMAL MATTERS:

Claim 27 was canceled without prejudice in the previous Response by the Applicants. Claims 16-24 are canceled herein without prejudice.

Claims 14 and 26 are amended. Support for the amendment to Claim 14 is found throughout the specification, for example on page 6, line 6. Claim 26 is amended to delete an element that is already a limitation in a claim upon which Claim 26 depends.

No new matter is added. As such, the Examiner is requested to enter the above amendments.

REJECTIONS UNDER §103(A)

Claims 14, 15, 25-32 are rejected under 35 USC 103(a) as being unpatentable over Frankel et a. (US Patent No. 6,099,848, issued August 8, 2000) in view of Frazao et al. (WO 99/07861, published 18 February 1999) and Loessner et al. (Molecular Microbiology 35(2):324-340, 2000).

In order to meet its burden in establishing a rejection under 35 U.S.C. §103, the Office must first demonstrate that a prior art reference, or references when combined, teach or suggest all claim elements. S*ee, e.g., KSR Int'l Co. v. Teleflex Inc.*, 127 S.Ct. 1727, 1740 (2007); *Pharmastem Therapeutics v. Viacell et al.*, 491 F.3d 1342, 1360 (Fed. Cir. 2007); MPEP § 2143(A)(1). In addition to demonstrating that all elements were known in the prior art, the Office must also articulate a reason for combining the elements. See, *e.g., KSR* at 1741; *Omegaflex, Inc. v. Parker-Hannifin Corp.*, 243 Fed. Appx. 592, 595-596 (Fed. Cir. 2007) citing *KSR*. Further, the Supreme Court in *KSR* also stated that "a court *must* ask whether the improvement is more than the predictable use of prior art elements according to their established functions." *KSR* at 1740; emphasis added . As such, in addition to showing that all elements of a claim were known in the prior art and that one of skill had a reason to combine them, the Office must also provide evidence that the combination would be a predicted success.

In the Applicants' previous response, the Applicants asserted that there was no credible reason to combine the cited references in the manner put forth by the Office, because one would not be inherently motivated by Frazao to modify Frankel to use an integrase and an integrase attachment site instead of homologous recombination, or further to look to Loessner for a Listerophage integrase and integrase attachment site in particular. To do so would require one to employ the more complicated two vector plasmid method of Frazao, and then further complicate this method by using the untried and untested Listeriophage A118 integrase and integrase attachment sites from Loessner. One of skill in the art looking to express polypeptides in Listeria need look no further than Frankel and thus would have no motivation to substitute Frazao's method from *M. Tuberculosis*, and then further modify that method with additional untried elements from Loessner in the hope that this would provide expression in Listeria equal to Frankel.

In maintaining this rejection, the Examiner asserts that this argument is not persuasive, because:

> Frazao et al. specifically teaches 'The scope of the present invention is not restricted to mycobacteria and is applicable to other bacteria such as: *Samonella* spp., *Vibrio* spp., *Shigella* spp., *Lactobacillus* spp., *Streptomyces* spp., *Corynebacterium* spp., *Listeria* spp . . . ' (page 7, lines 4-7). This is not persuasive because the office has recited a credible reason for combination and the art as combined merely substitutes one known element with the same function for another.

Furthermore, the Examiner has discounted the Applicants' position that integrase attachment sites are different from homologous recombination by stating:

> This is simply not true, because the integrase attachment sites provide for homologous recombination into the genome. These are not separate concepts in the art. In fact, as described by the cited art, the integrase sites allow site specific homologous recombination and the use of phage attachment sites for site-specific homologous recombination has been known for over 10 years.

Finally, the Examiner has discounted the Applicants' position that there would be no reasonable expectation of success that elements removed from their known sequence and placed in a vector would function the same way by stating:

> This is not persuasive because similar elements from a variety of phages have been used in site-specific integrative plasmid vector construction in the art, and thus, it would have been reasonable to expect that the same would be true for the sequences of Loessner et al. as particularly suggested by Frazao et al.

The Applicants respectfully maintain that there would be no credible reason to combine the elements of the cited references because one would not be inherently motivated by Frazao to modify Frankel to use an integrase and an integrase attachment site instead of homologous recombination, or further to look to Loessner for a Listerophage integrase and integrase attachment site in particular. Simply because Frazao teaches that it may be possible would not motivate one of ordinary skill in the art to do so, especially in light of the lack of support in the art for such teachings with regard to Listerophage integrase attachment sites.

Furthermore, the Applicants respectfully maintain that integrase-mediated site-specific recombination is different from homologous recombination. Integrase-mediated site-specific recombination is understood in the art to be the recombination between two DNA molecules that is mediated by a recombinase enzyme called an integrase (Lodish et al. (1995) Molecular Cell Biology, 3d Ed, p. 395, col. 1, l. 9-10; p. 395, col. 2, l. 1) (Exhibit A), which is responsible both for initiating (p. 396, col. 1, l. 5-8) and resolving (p. 396, col. 1, l. 14-17) the integration. The recombination event occurs at defined recombination sequences (p. 395, col. 1, l. 8-10), called phage attachment and integration sequences on the phage DNA and cell genomic DNA, respectively (see figure 10-35 for an example of integration of lambda phage). Accordingly, integrase-mediated site-specific recombination provides the benefit of recombination at a place along the DNA molecule that has been provided by the artisan, namely the site of the attachment or integration sequence.

In contrast, homologous recombination is the exchange of nucleotides between two homologous sequences following a break in the DNA. Unlike the requirement by integrase-mediated recombination for an enzyme to initiate the reaction, homologous recombination does

not require an enzyme for initiation (Lodish et al. (1995), see for example, recombination in E. coli, p. 392, col. 1, l. 14 - col. 2, l. 9), although one may be involved (p. 392, col. 2, l. 23-28). Furthermore, unlike the requirement by integrase-mediated recombination for a defined recombination sequence, homologous recombination can occur between any two homologous sequences (p. 389, col. 1, l. 11 – p. 395, col. 1, l. 3); thus, although the Examiner may maintain that homologous recombination is "site-specific" in the sense that it is specific to sequences of high homology, the Applicants submit that one of ordinary skill in the art would not understand homologous recombination to be "site specific" when examples exist in the art in which recombination is actually *restricted* to occur at a particular recombination sequence as it is in integrase-mediated site specific recombination.

To support the assertions that homologous recombination and site-directed integration are not distinct in the art, the Examiner recited the following passage from Frankel in the previous Office Action (Office Action April 3, 2008, p. 6, l. 4-8):

> A preferred method for producing recombinant Listeria having a gene encoding a heterologous antigen integrated into the chromosome thereof, is the induction of homologous recombination between a temperature sensitive plasmid comprising DNA encoding the antigen and Listeria chromosomal DNA. Stable transformants of Listeria which express the desired antigen may be isolated and characterized as described herein in the experimental examples. This method of homologous recombination is advantageous in that site directed insertion of DNA encoding the heterologous antigen is effected, thereby minimizing the possibility of disruption of other areas of the Listeria chromosome which may be essential for growth of this organism (column 8, l. 15-27).

Accordingly, Frankel et al. teaches that homologous recombination provides for site-specific integration. However, it is unclear from the passage if Frankel is teaching the kind of "site-specific" recombination provided by homologous recombination which does not require an enzyme or particular sequence for initiation, or the integrase-mediated site-specific integration taught by the pending claims. To understand Frankel's intent, one must turn to the art at the time of Frankel's invention. Like Frankel et al., Shen et al. (of the record) teach homologous recombination with temperature sensitive plasmids. Figure 1 of Shen et al., reproduced below, teaches that this homologous recombination event occurs between arms in the plasmid that are generally homologous to the Listeria chromosomal DNA with which they are recombining; furthermore, the figure does not teach that integrase activity is required:

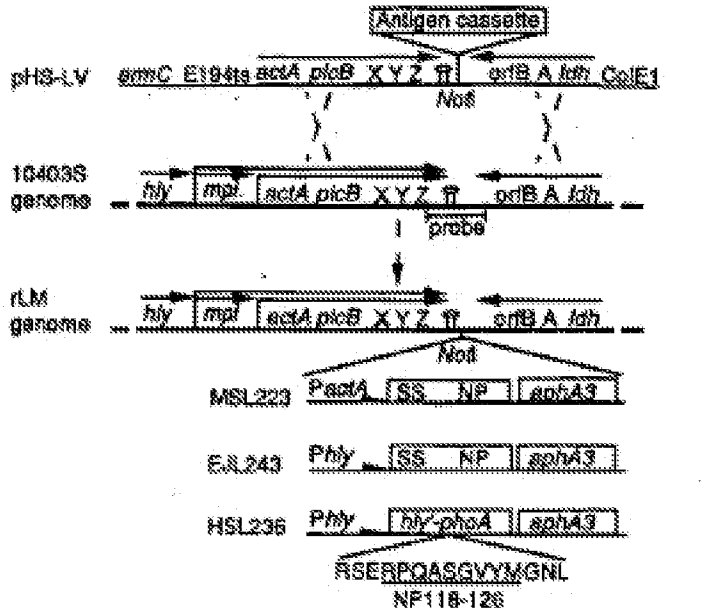

FIG. 1. Construction of recombinant LM (rLM) strains expressing LCMV antigens. The delivery vector, pHS-LV, carries (*i*) the *ermC* gene, conferring erythromycin resistance; (*ii*) the ColE1 origin of replication derived from pBR322; (*iii*) the thermosensitive origin of replication from pE194ts, which is conditionally functional in LM; and (*iv*) a 5.6-kb fragment of LM DNA with a *Not* I site introduced downstream from a transcriptional terminator (ΨΨ) between the lecithinase (*plcB*) and lactate dehydrogenase (*ldh*) operons. The *hly*, *mpl*, and *actA* genes encode LLO, a metalloprotease, and ActA, respectively, and loci designated X, Y, Z, orfB, and A are uncharacterized open reading frames (2, 17). Antigen expression cassettes are cloned into the *Not* I site of pHS-LV and integrated into the genome of wild-type strain 10403S by two homologous recombination events. Recombinant strains MSL223 and EJL243 have the LCMV NP gene fused to the signal sequences (SS) and promoters (P) of *actA* and of *hly*, respectively. HSL236 expresses the NP118–126 epitope within a *hly'-phoA* fusion. Antigen cassettes carry the *aphA3* gene, conferring kanamycin resistance.

Because Frankel et al. does not provide more guidance on the mechanism by which homologous recombination between a temperature sensitive plasmid comprising DNA encoding the antigen and Listeria chromosomal DNA occurs, one of ordinary skill in the art would extrapolate that the method taught by Frankel is the same as that taught by Shen et al., that is, not reliant upon the presence of listeriophage attachment sites or the integrase enzyme. Thus, Frankel does not teach or disclose site-specific Listeria genome integration of the pending claims.

However, in an effort to expedite prosecution and further distinguish the integration vector of the pending claim from one that relies upon homologous recombination, the Applicants

have amended to Claim 14 to recite "wherein said cells are transformed with an integration vector capable of integrase mediated site-specific Listeria genome integration, wherein said integration vector comprises a listeriophage attachment site", thereby clarifying that the site-specific recombination is mediate by integrase. The Applicants submit that Frankel clearly does not teach or make obvious this element.

Furthermore, Frankel et al. also does not teach or make obvious the element "wherein said cells are transformed with an integration vector capable of integrase mediated site-specific Listeria genome integration, wherein said integration vector comprises a listeriophage attachment site" (emphasis added) of the pending claims because Frankel does not teach the sequence of a listeriophage attachment site that, when placed on a DNA molecule, is sufficient to confer upon that DNA molecule the ability to undergo integrase-mediated recombination with another DNA molecule.

Loessner et al. teaches a putative listeriophage attachment sequence, identified *in silico* by its homology with attachment sequences of other phage. However, Loessner et al. does not teach that the putative listeriophage attachment sequence they have identified is either necessary or sufficient to confer upon a DNA molecule the ability to undergo integrase-mediated recombination with another DNA molecule. Loessner et al. has not taught that isolating the putative listeriophage attachment sequence from the complete phage genome and placing it on a vector will provide for successful site-specific integrase-mediated recombination, thus that it is necessary and sufficient to confer the ability to integrate in the presence of integrase. Until the Applicants demonstrated in the present invention that an isolated, cloned attP could confer upon the recipient DNA molecule the ability to recombine, one of ordinary skill in the art would have had no reasonable expectation that the attP would remain functional after separation from the flanking sequences. Thus, Loessner et al. does not teach nor make obvious the element of "wherein said cells are transformed with an integration vector capable of integrase mediated site-specific Listeria genome integration, wherein said integration vector comprises a listeriophage attachment site".

The Examiner states that Frazao et al. teaches that "the scope of the present invention is not restricted to mycobacterium and is applicable to other bacteria, such as Salmonella ssp., Vibrio, sppl, Shigella spp., Lactobacillus spp., Streptomyces spp., Corynebacterium spp., Listeria spp. . . . " (Final Office Action, p. 3, l. 5-8). The Applicants submit that Frazao et al.

teaches an attachment sequence for Mycobacterium that can be isolated and placed on a separate DNA molecule so as to confer upon that molecule the ability to recombine by integrase-mediated recombination, but provides only speculation that one exists for Listeriophage. For one of ordinary skill in the art to have any expectation of success in conferring upon a DNA molecule the ability to recombine by isolating the putative Listeriaphage attachment sequence taught by Loessner and cloning it onto the DNA molecule, one of ordinary skill in the art would need a working example of such success. Frazao et al. does not provide such an example. Therefore, Frazao et al. also does not teach or make obvious the element "wherein said cells are transformed with an integration vector capable of site-specific Listeria genome integration, wherein said integration vector comprises a listeriophage attachment site".

In light of the above remarks, reconsideration and withdrawal of the rejection is respectfully requested.

CONCLUSION

Applicant submits that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, please telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number BERK-017CIP.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: January 15, 2009

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Enclosure(s): Exhibit A- Lodish et al.

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